DECLARATION OF KELLY FLOOK, PH.D, UNDER RULE 132		
	Application No.	10/778,010
	Filing Date	02/11/2004
	First Named Inventor	Srinivasan
	Examiner Name	E. Therkorn
	Title	Ion exchange particle-bound flow-through porous monolith
	Attorney Docket No.	067407-5118US

1. I am employed by Dionex Corporation, assignee of the above application, in the position of Senior Chemist with a primary focus on development of monolithic chromatographic columns. My *curriculum vitae* is attached as Exhibit A.

2. I have reviewed the above patent application, the Office Action dated March 10, 2006, and the related prior art, particularly Sherrington U.S. Patent No. 5,066,784. I performed a series of experiments from which I conclude that the application claims are unobvious in view of such prior art.

3. In a first experiment, based on the disclosure of U.S. Patent No. 4,522,953, cited in the Sherrington patent, I made a polyHIPE chromatography monolithic column body as follows:

1. 0.70 g of potassium persulfate was dissolved in 350 mL deionized water.

2. 2.00 g of Span 80 was mixed with 10 mL styrene and 0.5 mL divinylbenzene (55% containing 45% ethylvinylbenzene) in a chilled (10°C) round-bottomed flask containing fitted with an overhead stirrer.

3. The potassium persulfate solution was added dropwise to the round-bottomed flask with stirring at 300 rpm.

4. After complete addition the mixture was stirred for a further 30 minutes.

5. A 4.6 x 50 mm column body (capped at one end) was filled with the formed HIPE using a syringe and piece of tubing.

6. The other end of the column was capped and placed in an oven at 50°C to polymerize for 24 hours.

7. The end caps of the column were replaced with fittings with through holes to

allow attachment to a syringe pump.

8. To wash unreacted components from the formed polyHIPE 20 mL isopropanol was flowed through the column at 0.25mL/min.

9. 20 mL water was flowed through the column at 0.25 mL/min.

10. 20 mL acetone was flowed through the column at 0.25 mL/min.

11. The column was then allowed to dry so that most of the acetone was removed.

4. Based again on the disclosure of Sherrington, I prepared an acrylamide solution for infusion into the monolith as follows:

1. 0.20 g AIBN initiator, 0.15g methylene bis acrylamide and 5.00g N,N-dimethyl acrylamide were dissolved in 10 mL 1,2-dichloroethane.

2. The solution was then purged with nitrogen gas to expel oxygen.

5. I filled the polyHIPE monolithic column with the acrylamide solution as follows:

1. A syringe pump was filled with the acrylamide solution.

2. The polyHIPE column was attached to the syringe pump.

3. 5 mL of the acrylamide solution was pumped through the column at 0.1 mL/min.

6. The acrylamide was polymerized as follows:

1. The polyHIPE column filled with acrylamide solution was sealed with end plugs and polymerized in a water bath at 60°C for 1 hour.

2. When polymerization was complete the column was removed and attached to a syringe pump containing dimethylformamide (DMF) which was pumped through the column at 0.1 mL/min to remove any unincorporated gel.

3. If the backpressure registered 2000 psi (upper operating limits for the pump) the flow was stopped.

7. The results of testing of the PolyHIPE column were as follows:

When the PolyHIPE column was washed with DMF at 0.1 mL/min the backpressure increased to >1000 psi. Small pieces of polymer were also observed extruding out of the end of the column into the waste beaker. After a short period of time the backpressure dropped significantly. Upon investigation the column was found to be empty of polymeric substrate. From this, I conclude that in a flow though format such as used in liquid chromatography, the polyHIPE column prepared was not structurally sound enough to withstand the swelling and washing process.

8. In a second experiment, a monolithic column (dimension 4.6 mm diameter, 50 mm in length) sold by Dionex Corporation under the ProSwift RP-1S trademark ("the Dionex monolith") was supplied to me. I am informed it was prepared using standard production procedures modified as follows. The one exception is that frits were not used because they possibly could block during the gel formation. Fittings were used with a through hole. (ref: 2006-032-016-06) These monoliths are typically 60% porous with a modal pore size of approximately 1 micron. The column was washed with acetone and allowed to dry so that most of the acetone as removed.

9. I filled the Dionex monolith according to the procedure of paragraph 5 and polymerized the acrylamide according to the procedure of paragraph 6.

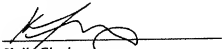
10. No flow of wash solvent (DMF) through the gel infused Dionex monolith could be established even when the flow rate was reduced to 0.05 mL/min. Typically, such a column would be operated for chromatographic applications at a flow rate of 1 mL/min or greater and the linear velocity under these conditions would be calculated as 0.1 cm/sec. (If flow would have been established at the flow rate of 0.05 mL/min this would translate into a linear velocity of 0.005 cm/sec which is too low to be practically useful for liquid chromatography.)

11. In contrast, another Dionex monolith was filled with latex particles generally according to the method described in the above patent application ("the claimed monolith"). At a flow rate of 0.015 mL/min, the back pressure was 900 psi, an acceptable flow rate and backpressure for chromatography. The backpressure of the uninfused Dionex monolith was substantially unchanged after infusion with the latex particles. The flow rate of 0.015 mL/min translates into a linear velocity of 0.5 cm/sec. The equivalent flow rate with a 4.6 mm x 50 mm column made by the present invention would be 5 mL/min. In contrast, no flow could be established in the gel infused monolith at 0.05 mL/min and at a backpressure

of 2000 psi, the upper pressure limit of the pump. In my opinion, this lack of flow is caused by the gel filling the pores of the monolith through which the solution would otherwise flow.

12. By way of summary, no flow of solution could be established in a gel-filled monolith column, even one using the Dionex monolith, and even at a backpressure substantially in excess of that used for liquid chromatography. In contrast, a monolith including the irreversibly bound fine ion exchange layering particles of the patent application claims has substantially the same backpressure as the empty monolith, and is perfectly suitable for high pressure liquid chromatography. In my opinion, this clearly establishes the superiority of the claimed invention over the cited prior art. Moreover, in my opinion the experiments show that a gel-filled monolith according to Sherrington, using a polyHIPE or Dionex monolith, was inoperable for use as a liquid chromatography medium.

I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature 

Kelly Flook

Date 3/27/07

Curriculum Vitae of Kelly Flook, Ph.D.

Experience

Oct '05 to present **Senior Chemist,** *Dionex Corporation, Sunnyvale, California*
Development of consumable products primarily the ProSwift monolithic line of analytical columns.

May '03 to Sept '05 **Postdoctoral Research Fellow,** *University of California, Berkeley, California*
Research and development of analytical techniques under the supervision of Prof. JMJ Fréchet and Prof. R. Mathies. Primarily the development of a multiplex device for the detection of infectious diseases (Mathies) and investigations into new matrices for matrix assisted laser desorption-ionization time-of-flight mass spectrometry (Fréchet).

Jan '00 to May '02 **Laboratory/Teaching Assistant,** *University of Durham, Durham, UK*
Supervisory laboratory and teaching assistant part-time while completing PhD research. Duties included laboratory set-up for undergraduate experiments in Organic, Physical, Analytical and Inorganic Chemistry. Supervision of students as well as teaching of the appropriate subject matter when required in groups or on a one to one basis.

July '99 to Oct '99 **Research Scientist,** *Proctor & Gamble Ltd, Newcastle upon Tyne, UK*
New product development, working within a close team of chemists at home and on other sites to develop novel product ideas to a stage implementable by the product development teams. Experience of formulation development and pilot plant scale up and co-ordination.

July '97 to Sept '98 **Research Scientist,** *Proctor & Gamble Ltd, Newcastle upon Tyne, UK*
A placement year developing 'blue-sky' chemistries into product ideas. Often required to multi task effectively and efficiently. Invaluable experience gained on the diversity of backgrounds required to carry out such a task. The ability to work effectively combining and expanding current knowledge to achieve the goals laid out by the project. Experience managing my own project and 'junior' group members.
Projects involved organic synthesis, HPLC, Capillary electrophoresis, NMR, method development.

Education

Oct '99 to May '03 **PhD Chemistry; University of Durham, Durham, UK**
Thesis title: Polymerised Microemulsions As Stationary Phases For Capillary Electrochromatography. Under the supervision of Dr. N.R. Cameron.
Microemulsion characterisation by NMR and conductivity measurements. Stationary phase preparation and analysis by mercury porosimetry and nitrogen adsorption. Capillary electrochromatography carried out using Beckman MDQ CE system.
Industrial Sponsor: AstraZeneca CASE Studentship. Including 3 months on-site research.
Awards: RSC Analytical Division bursary, RSC Macro Group award.

EXHIBIT A

Conferences: RSC Young Analysts Meeting, University of East Anglia, 2000; HPLC 2001, Kyoto, Japan, 2001; MicroScience 2002, Excel Centre, London, 2002; IRC Industrial Club Meeting, University of Bradford, 2002.

Sept. '95 to July '99 **BSc(Hons) Chemistry with Analytical Chemistry (1st Class); University of Northumbria at Newcastle, Newcastle Upon Tyne, UK**
Dissertation title: The Analysis of Flavones in Artists Pigments By Capillary Electrophoresis.
Several specialised modules involving theory and practical experience of chromatographic and spectroscopic techniques.

Sept '93 to July '95 **Chemistry, Maths, Physics A-level; Tynemouth College, North Shields, Tyne and Wear, UK**
Chemistry (B), Maths (B), Physics (C).

Publications

1. Polymerised bicontinuous microemulsions as stationary phases for capillary electrochromatography, N. R. Cameron, K. J. Flook and S. A. C. Wren, Chromatographia, **2003**, 57, (3-4) 203-206

2. Polymerised Bicontinuous Microemulsions as Stationary Phases for CEC: The effect of pore size on chromatographic performance. K. J. Flook, N. R. Cameron and S. A .C. Wren, J. Chrom A. **2004**, 1044 (1-2): 245-252

3. The influence of composition on the morphology of monoliths prepared from butyl methacrylate microemuslions. K. J. Flook, N. R. Cameron - Pending

4. Polymerisable microemulsions: The influence of microemulsion type on monolith topography at the air interface. K. J. Flook, N. R. Cameron - Pending

EXHIBIT A